

94/24160, and a figure showing the sequence alignment of human G-CSF and murine G-CSF, that are referenced herein.

REMARKS

***Withdrawn Objections and/or Rejections***

Applicants acknowledge that the rejection of claims 1<sup>a</sup>, 2, 5, 6, and 10-14 under 35 U.S.C. § 112, first paragraph, and the rejection of claim 1 under 35 U.S.C. § 112, second paragraph, are withdrawn in view of the amended claims.

***35 U.S.C. § 112, second paragraph***

The Examiner maintained the rejection of claims 4, 9, 11-14, as being unclear, under 35 U.S.C. § 112, second paragraph. **Applicants request reconsideration of the finality of the present Office Action because the Final Rejection is premature.** The Examiner failed to establish the clear issue of the rejection prior to the Final Rejection and the applicants has not been accorded the required opportunity to respond. At the Examiner's own admission in the present Office Action (Paper No.13, dated 14 October 1999) this rejection in the previous Office Action was unclear. The applicants made a good faith response to amend the claims in response to the Examiner's unclear rejection. Consequently, the Final Rejection is premature. **Applicants request reconsideration of the Final Rejection.**

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<sup>a</sup> The Examiner cited claim 11. Applicants believe that this is a typographical error and the Examiner meant to cite claim 1. Applicants have responded accordingly. If the applicant's assumption is incorrect, clarification is requested.

**35 U.S.C. § 103**

The Examiner maintained the rejection of claims 1, 5 and 10-14 under 35 U.S.C. § 103(a) as being unpatentable over Pastan *et al.* (U.S. Patent 5,635,599) in view of Lin (U.S. Patent 4,703,008). The Examiner maintains the argument that it would have allegedly been obvious to make circular permuted EPO molecules, from the suggestion made by Pastan ('599), having a breakpoint at non-conserved positions (positions 25, 27, 30, 32, 80, 82, 88, 95, 99, 105, 116, 121, 139 or 163) between two closely related EPO proteins ('008).

Applicants maintain the arguments in traverse of this rejection, set forth in the response dated 09 February 1999 and 30 August 1999, that the Examiner has failed to establish a *prima facie* case of obviousness.

The Office has failed to establish a *prima facie* case of obviousness because the Examiner has completely ignored the primary requirement set forth by Pastan ('599). Pastan ('599) **requires** that the opening site does **not** interrupt protein folding. The '599 application states that:

*Circular permutation **requires** that a protein have an opening site (i.e., between residues n and N + 1) where the formation of the termini will **not** interrupt secondary structure crucial in the folding process or*

***critical elements of the final conformation.***

*Even if the three-dimensional structure is compatible with joining the termini, it is conceivable that the kinetics and thermodynamics of folding would be greatly altered by circular permutation if opening the circularized protein separates residues that participate in short range interactions crucial for the folding mechanism or the stability of the native state. Goldenberg, Protein Eng., 7:493-495 (1989). Thus the choice of the opening site is important to the protein activity.* (paragraph bridging bottom of column 7 and top of page 8, emphasis added).

The unpredictable nature of circular permutation and the impact on protein folding has been well documented in the art. Goldenberg (1989) states:

*However, the techniques of genetic engineering make it possible to circularly permute any DNA sequence and, therefore, any polypeptide sequence. Whether the resulting protein will fold, however, is not assured.* (page 493, column 1, 2<sup>nd</sup> paragraph, emphasis added).

The Examiner has focused on a single consideration for selecting breakpoints suggested by Pastan (i.e. that non-conserved regions can be permuted) but has ignored the fact that Pastan **requires** that it does not interrupt protein

folding. Pastan says that circular permutation **requires** that the selection of the breakpoint does not interrupt secondary structures that are **crucial** for proper protein folding or **critical** elements of the final conformation. Pastan ('599) and Lin ('008) are silent on the protein folding of EPO. Therefore, the Examiner has failed to establish a *prima facie* case of obviousness because the Examiner has failed to establish that the prior art teaches the secondary structures **crucial** for proper protein folding or the **critical** elements of the final conformation of EPO. Therefore, an undue burden of experimentation is required to determine what regions are **critical** for protein folding.

The applicants once again point to the entirety of the prior art on circular permutation, which factually establishes that circular permutation is unpredictable. . The '599 file history affirms that the circular permutation of any given ligand is unpredictable. The applicant states:

*Moreover, absent a showing that a particular ligand (e.g. IL-2, IL-4, G-CSF or GM-CSF) retains activity when it is circularly permuted, there is no a priori bases to expect that a particular ligand is suitable for circular permutation. (page 17 of the response filed April 8, 1994)*

The applicant's own statement confirms that it is unpredictable if a ligand (e.g. EPO) is suitable for circular permutation. The Examiner's own rejection in '599 that the art is unpredictable further supports the applicants position. Two working examples of circular permutations of IL-4 in '599 do not instantly reverse the

predictability of circular permutation of other ligands (i.e. EPO).

At best the prior art merely invites further experimentation, i.e., the present rejection is based upon the repeatedly rejected improper standard of **obvious to try**. *In re Mercier*, 185 USPQ 774 (CCPA 1975); *Ex parte Old*, 229 USPQ 196 (BPAI 1985); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81 (Fed. Cir. 1986); *In re Geiger*, 2 USPQ2d 176 (Fed. Cir. 1987); *In re Dow Chemical Co.*, 5 USPQ2d 1529 (Fed. Cir. 1988); *In re O'Farrell*, 7 USPQ2d 1673, 1680 (Fed. Cir. 1988).

As explained in *O'Farrell* at 1681, the admonition that **obvious to try** is not the standard under § 103 has been directed mainly at two kinds of error:

1. Varying all parameters or trying each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were **critical**, or no direction as to which of many possible choices is likely to be successful; and

2. Exploring a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

The Examiner's *prima facie* case of obviousness is improper because both of the situations of *O'Farrell* apply here.

***The present situation is exactly the first error that the Federal Circuit, in O'Farrell, exhorted was an improper***

**obvious to try situation and is not the standard of obviousness under 35 U.S.C. § 103(a).**

The applicants pointed out that a number of general statements are made by Pastan ('599) as to selecting sites, which Pastan characterizes as **good** candidates for breakpoints. Applicants have summarized these general considerations for the selection of "opening sites" (last paragraph of column 7 through first paragraph of column 9); 1) that the opening site is in a region that lacks structure; 2) that the opening site is at a residue that can be substituted; 3) that the opening site is at a residue that can be modified (ie. glycosylated); and 4) that the opening site is in a non-conserved region amongst a related family of proteins. However, Pastan ('599) has only suggested varying a number of parameters without indicating which are **critical**.

Pastan ('599) only teaches that in the case of IL-4 that residues 38 and 105 are potential glycosylation sites and that circular permutation at these two breakpoints results in molecules that maintain only partial bioactivity compared to native IL-4.

There is no factual basis or experimental data in '599 supporting the generalization that opening sites can be made in regions that lack structure. There is no factual basis or experimental data in '599 supporting the generalization that opening sites can be made at sites that can be substituted. There is no factual basis or experimental data in '599 supporting the generalization that opening sites can be made in non-conserved regions.

The lack of data or experimental evidence in the Pastan ('599) specification is convincing factual evidence that these are unsupported speculations. These speculations do not provide guidance because they fail to identify what parameters are **critical** but rather are only a listing of possibilities.

However, perhaps nothing is more telling as to Pastan's ('599) total lack of guidance than in the section suggesting that non-conserved regions between a family of proteins, which is relied upon by the Office in attempting to establish a *prima facie* case of obviousness. Pastan states:

**Preferred** opening sites are then selected in regions of the protein that **do not show highly conserved** sequence identity between various members of the protein family. **Alternatively**, if an opening site is identified in a **conserved region** of the protein, that **same region provides a good candidate** for opening sites in a homologous protein. (column 8, lines 45-53) emphasis added

Such admissions to try one parameter and if that does not work then do just the opposite, can not be construed as teaching which parameters are **critical**. The circular permutation prior art, specifically Pastan ('599), does not provide the level of teaching that is required under the obviousness standard. If merely listing compounds, as in case in '599 where other cytokines including EPO and possible breakpoints are only listed, could suffice as a disclosure, it would bar patent protection to the person who actually discovered a compound on the list, and in so doing, thwart the Constitutional purpose of the patent system.

Even if the speculations made by Pastan are accepted as providing guidance, the Examiner has failed to establish that there is a reasonable expectation of success. Pastan states that the suggested selections make **good** breakpoints. Pastan characterization of these selections as **good** indicates to one skilled in the art that there is a **high probability** that these selections will work, not merely a crap-shoot.

The Examiner relies on Lin ('008) to provide a sequence alignment of two EPO species, human and cynomolgus monkey. The Examiner argued that the breakpoints at non-conserved position (25, 27, 30, 32, 80, 82, 88, 95, 99, 105, 116, 121, 139 or 163), as determined from the alignment of the two sequences, were obvious.

As the applicants have previously pointed out, a homology alignment of only two species constitutes a very narrow comparison. A much more robust sequence alignment of the "family of EPOs" is presented in Figure 6 of WO 94/24160 (of record). A much different picture is apparent from the comparison of seven species in WO 94/24160 (applicants have provided an annotated copy of Figure 6) versus the two species from '008. Several differences can be identified. First, at 4 positions (27, 82, 139, and 163), out of the 14 non-conserved positions between human and cynomolgus monkey, the amino acid is conserved in every species except human. Second, at 4 positions (30, 80, 104, and 116), out of the 14 non-conserved positions between human and cynomolgus monkey, the amino acid is conserved in every species except the two Macaca species. Third, at 2 positions (99 and 121), out of

the 14 non-conserved positions between human and cynomolgus monkey, either the human or monkey amino acid is conserved in the other species. Forth, at 2 positions (25 and 32), out of the 14 non-conserved positions between human and cynomolgus monkey, the 'monkey' amino acid or a single other amino acid is conserved in every species except human. Fifth, at 1 position (95), out of the 14 non-conserved positions between human and cynomolgus monkey, the human amino acid is conserved in three other species. Sixth, at 7 positions (25, 30, 80, 82, 95, 99, 105), out of the 14 non-conserved positions between human and cynomolgus monkey, the changes(s) are conservative amino acids substitutions. Seventh, there are 47 additional positions, which are not conserved between all seven of the species.

The factual analysis of the more robust homology comparison (seven species vs. two) shows that "EPO" is even more highly conserved amongst the seven species than the simple alignment of human and gibbon would indicate. "EPO's" high degree of cross-species sequence conservation, 80% - 92% between human EPO and other species, and bioactivity is atypical compared to 50% - 70% (Scheerlink, J-P. Y., *Veterinary Immunology and Immunopathology* 72:39-44, 1999 - copy enclosed for the examiner's convenience) of other 4  $\alpha$ -helical bundle cytokines (ie. IL-2, IL-4, GM-CSF and G-CSF). The lack of genetic diversity of EPO, between species, suggests to one skilled in the art that "EPO" would be a **poor** candidate for circular permutation.

The alignment of the seven EPO species also shows there are several inconsistencies between the different

speculations, made by Pastan ('599), regarding the selection of breakpoints in EPO.

First, Pastan on one hand suggests that glycosylation sites are good candidates for breakpoints and on the other hand non-conserved positions are good candidates. However, the potential glycosylation sites in EPO (24, 38, and 83) are conserved amongst the seven species (Figure 6 of WO 94/24160). So which suggestion is correct?

Second, Pastan on one hand suggests that non-structured regions between a family of closely related family members are good candidates for breakpoints and on the other hand non-conserved positions are good candidates. However, 8 positions (25, 80, 82, 95, 99, 105, 116 and 139), out of the 14 non-conserved positions between human and cynomolgus monkey, are in structured regions. So which suggestion is correct? The crystal structure of erythropoietin N24K, N38K, N83K, P121N, P122S has been solved to 1.9 Angstroms, refined to an R-factor of 24.2% (Syed, R. S. et al., *Nature* 395:511-516 (1998) - applicants have provided the Examiner with a copy) and deposited in the Brookhaven Protein Databank as PDB-ID:1EER. EPO has an up-up-down-down four helical bundle topology with interhelical angles similar to those of the long-chain class, for example G-CSF. However, it also contains two small antiparallel  $\beta$  strands typical of short-chain class, for example GM-CSF, IL-2 and IL-4. One pair of antiparallel long helices,  $\alpha$ A (residues 8-26) and  $\alpha$ D (residues 138-161) is held together by a disulfide bridge, Cys 7 to Cys 161. The other pair,  $\alpha$ B (residues 55-83) and  $\alpha$ C (residues 90-112), is linked by a short loop. The short segments of amino acids from the long AB and CD

crossover loops interact with each other to form an antiparallel  $\beta$  sheet  $\beta$ 1 (residues 39-41) and  $\beta$ 2 (residues 133-135). EPO has two additional short helicies, the  $\alpha$ B' (residues 47-52) and  $\alpha$ C' (residues 114-121). EPO forms a 1:2 complex with its receptor. Fig 1a & b (submitted herewith) shows two orientations of the corresponding ribbon diagrams of EPO with the EPO receptor. One copy of the receptor is drawn in purple, the second in blue. Erythropoietin is drawn with color coordinated helices so connectivity is readily discernible. As per the crystallographers' assignment helix 1 (white) consists of residues 9-26, helix 2 (yellow) of residues 56-82, helix 3 (emerald green) of residues 91-111 and helix 4 (red) of residues 138-161. Segments connecting the main helices of the bundle appear as blue-green ribbons. The N- and C-termini are distinguished, respectively, by white and red C-alpha CPK's.

Helix 3 docks against one copy of the receptor. Residues of erythropoietin helix 3 and receptor residues that are in close contact (<3.5 Angstroms) are shown in Fig. 2 (submitted herewith). The essential role of the third helix of EPO in receptor binding has been established (Groberg, J. et al. *Eur. J. Biochem.*, 218:597-601, 1993 - copy provided for the Examiner's convenience) Permutein breakpoints within helix 3, including the conserved residues 95, 99 and 105 (emerald green), are expected to produce molecules, which cannot bind both copies of the receptor analogously to native and, therefore, will not signal. Therefore, one skilled in the art is left guessing if conserved residues in EPO are **good** candidates or not.

This inconsistency between the different suggestions does not provide a high expectation, let alone of reasonable expectation of success. A listing of conflicting suggestions, such as in '599, does not provide one skilled in the art guidance but to the contrary presents an invitation to experimentation.

The Examiner contends that the suggestions Pastan ('599) made regarding the breakpoints were shown to be valid with respect to the IL-4, IL-2, G-CSF, and GM-CSF permuteins. While it is true that claims are to be held as valid it is also a fact that the claims do not include EPO. The applicants respectfully submit that the broad brush that the Examiner uses as to the scope of the enablement of the '599 specification is untenable. In light of the above mentioned facts regarding the lack of guidance and lack of predictability for selecting breakpoints in EPO, a *prima facie* case of obviousness has not been established.

In the present Office Action the Examiner also states that Lin ('008) is **not being relied upon to teach EPO analogs**. However, this statement appears to be in conflict with the Examiner's previous statement:

*"Lin discloses at column 11 that synthetic sequences that are partially duplicative of any of the two naturally occurring sequences could be made that retain activity." (page 7, lines 13-15 of Paper No.9)*

The Office's reversal appears to indicate that the Office has conceded that '008 does not enable EPO variants.

As previously pointed out by the applicants, the human EPO and monkey EPO sequences differ by fourteen amino acid substitutions. The Examiner has failed to provide a factual basis that any EPO analog with only one of the fourteen substitutions at a non-conserved position between human and cynomolgus monkey EPO. One skilled in the art knows that in general that some protein analogs can be made without impacting activity. However, the present situation is different because fourteen specific differences are together in a single protein and no factual basis has been established that any one of those positions can be substituted in the human EPO molecule without effecting activity. An example of a single substitution at a non-conserved position between two members of a related family of proteins that results in a significant loss in activity is the substitution of Asp<sup>112</sup> to Ala<sup>112</sup> in G-CSF (Young et al. *Protein Science* 6:1228-1236 – for the Examiner's convenience applicants have provide a copy). The sequence alignment of human G-CSF and murine G-CSF (provided herewith) shows that position 112 (145 including the leader) is non-conserved. When position 112 in human G-CSF is substituted with Alanine the proliferative activity is dramatically reduced 16 fold (Table 1 on p. 1229 of Young et al.). Hence, the Examiner's assertion that it is accepted that a single substitution at a non-conserved position can be substituted without effecting activity is not factually based.

The guidelines set forth by Pastan, for the selection of **good** candidates for breakpoints are not based on factual proofs. To the contrary they are only speculative and offer no guidance what so ever fit for generalization. The

patentee ('599) has only listed broad and general suggestions that propose virtually every single amino acid position as a **good** breakpoint candidate.

Thus, where the prior art (such as '599 in this case) does not indicate which parameters are **critical** and does not provide direction as to which of many possible choices is likely to be successful, the fact that a claimed invention falls within the scope of possible combinations considered therein does not render the invention unpatentably obvious.

***The present situation is exactly the second error that the Federal Circuit exhorted, in O'Farrell, was an improper obvious to try situation and is not the standard of obviousness under 35 U.S.C. § 103(a).***

The Examiner has improperly rejected the claims on the basis of the existence of a general method of making circular permuteins of '599 which is irrelevant as to the question whether the specific molecules would have been obvious. *In re Bell*, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Deuel*, 34 USPQ2d 1210, 1216 (Fed. Cir. 1995).

The Examiner contends that it is important to realize that Pastan ('599) patent was the first to **claim** circular permuteins of any protein. However, this is irrelevant because the prior art (see pages 6-10 of the present specification) provides a number of references which describe circular permutation as far back as 1983 (Goldenberg and Creighton *J. Mol. Biol.* **165**:407-413) in which the authors describe the *in vitro* circular permutation

of bovine pancreatic trypsin inhibitor. The prior art also discloses a general recombinant method to create circular permuted proteins was developed by Horlick in 1992 (Horlick et al., *Protein Eng.* **5**:427-431).

What is important to realize is the parental IL-4/exotoxin fusion has **only** <1% (0.14 nM/18 nM) of the binding affinity of native IL-4 and the IL-4(38-37) permutein/exotoxin has **only** 10% (0.14 nM/1.4 nM) of the binding affinity compared to native IL-4 (column 23, lines 42-59 and Fig 4a). Even if one looks at the proliferative activity of the two exemplified I-L4 permuteins (38-37 & 105-104) they have only 50% (1.2 nM/2.4 nM) and 60% (1.2 nM/2 nM) activity respectively compared to native IL4 (column 23, lines 4-14, and Fig 3b). Horlick et al. teaches that an IL-1 $\beta$  permutein (65/64) has nearly identical receptor binding activity compared to native IL-1 $\beta$  (p. 430 Fig 5).

Circular permutation is such a general method and exploring a new technology that seemed to be a promising field of experimentation, where the prior art gave only general guidance, as is the case with Pastan, as to the particular form of the claimed invention or how to achieve it is not the standard of obviousness.

In conclusion, the applicants respectfully submit that the Office has failed to establish a *prima facie* case of obviousness because the Examiner has failed to establish the critical protein folding elements for EPO, the generalizations made by Pastan are not factually supported and of success, the scope of the enablement of '008 is

limited to the two species disclosed, and '599 is not enabled beyond the scope of the claimed molecules. Therefore, it has **not** been established that '599 and '008 suggests the presently claimed molecules and does not establish a reasonable expectation of success. The applicants submit that the rejection is moot.

**35 U.S.C. § 103**

The Examiner maintained the rejection of claims 1-4 and 6-9 under 35 U.S.C. § 103(a) as being unpatentable over Pastan *et al.* (U.S. Patent 5,635,599) in view of Lin (U.S. Patent 4,703,008) and further in view of Chaudhary *et al.* (1989, *Nature* 339:394-397) and Cousens *et al.* (U.S. Patent 4,751,180). The Examiner states that '599 and '008 do not teach the GlySer rich linker required by claims 2-4 and 6-9. The Examiner argues that it would have been obvious to use the GlySer-rich linker for connecting antibody variable domains, as disclosed by Chaudhary and that non-polar amino acids are useful for a flexible linker, as disclosed by Cousens.

For the same reasons set forth above regarding '008 and '599, applicants submit that the rejection is moot.

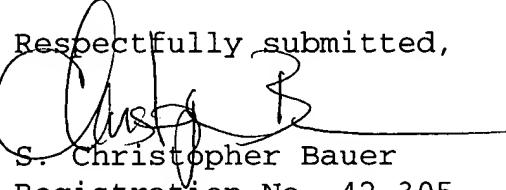
With respect to Chaudhary *et al.* and Gearing *et al.* ('180) applicants maintain the previous arguments that the requirements of linkers for joining fusion proteins are different from the requirements of the linkers for joining the ends of circular permuted molecules. Chaudhary *et al.* and

Gearing et al. ('180) do overcome the shortcomings of '599 and '008.

Claims 1-22 are pending and claims 15-22 are withdrawn from consideration.

**Applicants request reconsideration of the finality of the last Office because the Final Rejection is premature.**

Reexamination and reconsideration of the application as amended are requested. Allowance of the pending claims at an early date is solicited.

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